Are Environmental Sentinels Signaling?

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There is an increasing perception that environmental contamination by chemicals no longer poses a significant health threat and that relaxation of environmental regulations is warranted. However, many wildlife populations are showing signs of developmental, behavioral, and reproductive dysfunction due to environmental contamination by endocrine-disrupting chemicals. Scientists, regulators, and legislators must mobilize to identify current health threats posed by environmental pollutants, develop testing protocols that will detect such properties of new chemicals, and strengthen legislation designed to protect environmental health. *Key words*: chronic toxicity, endocrine disrupters, environmental sentinels, pesticides, reproductive toxicity. *Environ Health Perspect* 103:888–890 (1995)

The year 1995 marked the 25th anniversary of both the U.S. Environmental Protection Agency and Earth Day. The inception of both institutions signified the need to temper anthropogenic stresses on the environment or face unsettling consequences. Decades of environmental abuse culminated in the 1960s when public perception of the repercussion of unabated environmental pollution was heightened by Rachel Carson's graphic depictions (*1*). The pressing environmental problems of 25 years ago were blatant. Among the most significant of problems were chemical and sewage discharges making aquatic resources unsuitable for human use and habitation by aquatic organisms, and the use of pesticides, which posed a significant threat to nontarget species. In response, the Clean Water Act was instituted in 1972 to regulate waste discharge and to ensure that high water-quality standards were maintained. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was amended three times during the 1970s to provide safeguards against pesticide-mediated harm to human and environmental health. Such legislation provided the foundation upon which a sound and reasonable national environmental policy was established. This policy has resulted in significant improvement in environmental quality concurrent with population and economic growth. The success of the environmental protection policies of the United States is best exemplified when environmental quality of the United States is compared to that of industrialized countries of the former Soviet block and other countries where such policies were never significantly instituted (*2–4*).

With the current movement toward the reduction of government size and spending, the issue is often raised as to whether environmental legislation and supporting research programs could be relaxed without intolerable consequences. Major fish and wildlife kills due to chemical waste discharge and improper pesticide usage are now largely relegated to distant memory. If one accepts the thesis that fish and wildlife species serve as sentinels for the protection of human health from environmental contaminants, then human health must also be adequately protected from the adverse health effects of pollution. Such logic supports the contention that environmental legislation and research need not be expanded and could perhaps be relaxed. However, not factored into this argument is that, while the flagrant environmental problems of 25 years ago have been addressed, more subtle, though no less beguiling, environmental threats may persist. Central to this issue is the question, are environmental sentinels currently signaling the existence of such environmental hazards?

Toxicant-mediated endocrine disruption is one example of a toxicological hazard currently presenting itself in the environment. Endocrine-disrupting effects of environmental pollutants were first recognized while investigating mechanisms responsible for reproductive failure among some bird species exposed to organochlorine pesticides (*5*). The observation that exposure to some chemicals can lead to reproductive failure led to the promulgation of regulations under FIFRA and subsequently expanded to nonpesticide chemicals under the Toxic Substances Control Act requiring that the effects of chemical exposure on the production of viable offspring be determined. Such tests, conducted in standard test species of birds, fish, mammals, and invertebrates, involve chronically exposing the parent organisms to various concentrations of a chemical, then assessing the number of viable offspring produced (*6*). With fish, only subchronic testing, involving the assessment of the effects of the chemical on survival and growth of larval fish, is initially required. Assessments of reproductive toxicity are mandated only if the no-observed-effect level established during the subchronic toxicity test is greater than 1/10 the expected environmental concentration of the chemical (*6*). Retrospective assessments have shown that such approaches will adequately protect the environment against most chemicals (*7*). However, unique toxicological properties of some chemicals can result in undetected toxicity using these protocols. Endocrine-disrupting chemicals can be among these undetected toxicants because they may 1) elicit effects on the developing fetus that are not manifested until the mature organism enters its reproductive stage, 2) elicit specific biochemical/physiological changes that affect an organism's reproductive capacity without affecting survival and growth as measured during subchronic testing, or 3) adversely affect endocrine processes characteristic of some species but absent in those surrogate species used in toxicity testing. Many pesticides, industrial chemicals, and wastes are among the toxicants that elicit such effects.

Shore birds such as gulls and terns typically produce broods of two or three eggs. Ornithologists began observing in the 1970s that broods of five or six eggs were not uncommon (*8*). This abnormal clutch size was found to be due to multiple females sharing a nest (*9*). This female–female pairing appeared to be due to a deficiency in reproductively competent males (*10*). Laboratory investigations demonstrated that exposure to DDT feminized male gulls during embryonic development (*11*). Further, incidence of female–female pairing was higher in environments with significant DDT contamination (*10*). Thus, abnormal breeding behavior in these birds appeared to be due to reproductive deficiency in males caused by embryonic exposure to environmental pollutants. This observation is not only of historical relevance, as female–female pairing of terns has been noted recently in areas contaminated with polychlorinated biphenyls (PCBs) (*12*).

Female Poeciliidae fish inhabiting areas receiving pulp mill effluent have been observed to undergo masculinization. Most obvious is the modification of the anal fin in

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affected females to a gonopodiumlike structure (used by males for sperm transmission) (*13*). Exposure to exogenous androgens has been shown to cause similar masculinization (*14*), and androgens generated by the action of bacteria on phytosterols present in the effluent are presumed to be responsible for this effect. Fish exposed to paper and pulp mill effluent can also experience altered steroid hormone titers (*15*), impaired gonad development (*16*), and reduced fecundity (*17*). Such effects, specific to reproduction, would not be detected in subchronic toxicity tests.

Propiconazole is a member of the imidazole-derivative class of fungicides. A common characteristic of these chemicals is their ability to inhibit enzymes responsible for steroid hormone biosynthesis and induce enzymes involved in steroid hormone metabolism (*18,19*). A consequence of this effect is severe reductions in some steroid hormone levels (*18*). This specific and potent effect has led to the consideration of some imidazole-derivatives for use as a male contraceptive (*20*). Propiconazole, which is used as an agricultural fungicide, shares these properties and thus has the potential to compromise reproductive success of chronically exposed organisms. These effects would not be detected in a subchronic toxicity test that did not evaluate reproduction. The Ecuadorian shrimp industry has called for a moratorium on the use of propiconazole for fear that it is responsible for the demise of shrimp populations (*21*).

Tributyltin has been used extensively for more than 20 years as an antifoulant in marine paints. Tributyltin has been identified as the causative agent responsible for imposex in many marine mollusk populations. Imposex is the imposition of sex characteristics of one gender onto another (a form of pseudohermaphrodism). In the case of tributyltin-exposed mollusks, females develop a penis, vas deferens, and in severe cases, seminiferous tubules (*22*). Affected females can be rendered infertile because the vas deferens blocks the release of eggs from the oviduct. The mechanism responsible for this effect has not been conclusively established, but it seems to involve the neuroendocrine regulation of sexual differentiation (*23*). Tributyltin can cause imposex at low part per trillion concentrations and has caused the extinction of some affected populations (*22*). Certain mollusk species may be particularly sensitive to the effect of tributyltin owing to unique aspects of sexual differentiation in these organisms (*24*). Intersexuality also has been observed in some crustacean populations in the vicinity of sewage discharge, though causality has not been established (*25*). Peri- and neonatal exposure of rodent models to a variety of environmental chemicals including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*26*), PCBs (*27*), mirex (*28*), chlordecone (kepone) (*29–31*), dieldrin (*28*), aldrin (*28*), chlordane (*32*), and atrazine (*33*) have shown that these chemicals are capable of eliciting a variety of perturbations in the sexual differentiation of mammals.

Thus, it would appear that environmental sentinels are indeed signaling us that all is not well. Although the major environmental problems of the 1960s may have been successfully dealt with, we are faced in the 1990s with new problems to surmount. Speculation remains as to whether human heath issues such as increased incidence of breast cancer, prostate cancer, testicular cancer, endometriosis, birth defects in the male reproductive tract, and reductions in sperm count may be associated with the existence of endocrine-disrupting chemicals in the environment (*12*). Toxicity testing requirements for environmental chemicals must be expanded to consider effects that may go undetected using current guidelines. Existing toxicity testing requirements should be complemented with *in vitro* diagnostic tests designed to detect specific biological properties such as hormone agonistic or antagonistic activities. As discussed by McLachlan (*34*), the establishment of cell lines that have been transfected with specific receptor-reporter gene constructs would greatly facilitate the screening of chemicals for such properties. In addition, biomarkers must be identified that can be used as part of standard toxicity tests to identify chemicals that may pose risk of endocrine-disrupting effects. For example, a significant correlation has been shown between the percentage of males present in a litter of mice and the average anogenital distance in females in that litter (*35*). The intrauterine position of a female rodent with respect to the number of adjacent male siblings affects reproductive physiology and behavior (*36*) as well as anogenital distance (*37*). These observations suggest that the intrauterine hormonal environment affects the developmental and reproductive capacity of the offspring. Analyses of anogenital distance during conventional toxicity tests may thus serve as a biomarker of reproductive effects and as an indicator of the need for multigenerational toxicity tests. Production of estrogen-regulated proteins such as vitellogenin (*38*) and lactoferrin (*39*) in chemically exposed males would signal estrogenicity of the chemical, which would warrant more definitive testing protocols to explicitly characterize toxicity.

Changes in steroid hormone levels can also be indicative of endocrine-disrupting chemical exposure. Several studies have suggested that toxicant-induced alterations in steroid hormone levels or metabolism may contribute to reproductive impairment (*40–42*). Our laboratory has been conducting comparative studies of the effects of chemicals on reproductive capacity and steroid metabolism using the freshwater crustacean *Daphnia magna* in an attempt to validate this putative relationship. Experiments thus far indicate that concentrations of the toxicants that impair reproduction also perturb steroid metabolism (*43*). Furthermore, this effect on steroid metabolism can be detected after short-term exposure to the toxicant (*44*). These results suggest that for some reproductive toxicants, effects on steroid metabolism may be predictive of reproductive toxicity, and thus metabolic effects can serve as a biomarker of reproductive toxicity.

Clearly, many strategies exist that could improve our ability to detect endocrine-disrupting chemicals and identify exposure dosages at which effects are elicited. Further research is needed to better define such experimental approaches and validate their utility. Ultimately, testing requirements will need to be expanded to ensure the detection of endocrine-disrupting effects of environmental chemicals; environmental legislation must be strengthened to ensure protection against these and other chemicals that elicit subtle, yet devastating, effects. Legislators must be made aware that the absence of dead fish and wildlife is not justification for the relaxation of environmental legislation and supporting research. The deleterious consequences of chemicals in the environment continue. You just have to look a little harder to see them.

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